b) a focusing device for focusing said laser beam onto the measuring volume wherein the laser beam is such highly focused within the measuring volume that in essence it exclusively covers the measuring volume,

c) a detector device for detecting fluorescence radiation generated due to laser light excitation of one or more molecules, molecular complexes or molecular fragments in said measuring volume,

d) a pinhole aperture arranged in an image plane within a beam path of fluorescence radiation confocally with respect to the measuring volume to limit the quantity of fluorescence radiation to be detected by the detector device,

wherein the measuring volume is distanced from the focusing device by up to 1,000 µm.

60
~~120~~. A device for detecting one or more molecules, molecular complexes or molecular fragments in a measuring volume by means of fluorescence spectroscopy which device comprises:

a) a laser beam generation device for generating a laser beam with a first wavelength,

b) a focusing device for focusing said laser beam onto the measuring volume wherein the laser beam is such highly focused within the measuring volume that in essence it exclusively covers the measuring volume,

c) a detector device for detecting fluorescence radiation generated due to laser light excitation of one or more molecules, molecular complexes or molecular fragments, said detector device being arranged in an image plane within a beam path of fluorescence radiation confocally with respect to the measuring volume to limit the quantity of fluorescence radiation to be detected,



87

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wherein the measuring volume is distanced from the focusing device by up to 1,000 micrometer.

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121. The device according to claim 119 wherein said pinhole aperture has a diameter of ≤ 100 micrometer, preferably of ≤ 20 to 30 micrometer.

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122. The device according to claim 119 wherein said molecules, molecular complexes or molecular fragments are in a dilution of ≤ 1 micromolar.

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123. The device according to claim 119 wherein said measuring volume is ≤ $10^{-14}$ liter.

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124. The device according to claim 119 wherein with an image scale of 1:100, 1:60, or 1:40 between the measuring volume and the image plane and with a measuring volume having dimensions of ≤ 0.1 micrometer in each direction, said pinhole aperture has a diameter of about 10 micrometer, 6 micrometer, or 4 micrometer, respectively.

6

125. The device according to claim 119 wherein the focusing device for focusing said laser beam onto said measurement volume has a numerical aperture of ≥ 1.2 N.A.

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126. The device according to claim 119 wherein the focusing device has a prefocusing device to prefocus the laser beam and a focussing objective lens to focus the prefocused laser beam onto the measuring volume.



88

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8 ~~127~~. The device according to claim ~~126~~ wherein the distance between the focussing objective lens and the measuring volume is up to 1,000 micrometer.

9 ~~128~~. The device according to claim ~~126~~ wherein a semitransparent mirror is arranged between the prefocusing device and the focusing objective lens to deflect the prefocused laser beam on the focusing objective lens.

10 ~~129~~. The device according to claim ~~128~~ wherein the pinhole aperture is arranged at a side of the semitransparent mirror facing away from the focusing objective lens.

13 ~~130~~. The device according to claim ~~119~~ wherein an optic for transmitting the fluorescence radiation has a numerical aperture of ≥ 1.2 N.A.

14 ~~131~~. The device according to claim ~~119~~ wherein the detector device has at least one and preferably more detectors for detecting the fluorescence radiation.

15 ~~132~~. The device according to claim ~~119~~ wherein at least one optical filter is arranged between the pinhole aperture and the detector device.

16 ~~133~~. The device according to claim ~~119~~ wherein at least one imaging lens is arranged between the pinhole aperture and the detector device.



89

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17 ~~134~~. The device according to claim ~~119~~ 1 wherein at least one semitransparent mirror is arranged between the pinhole aperture and the detector device.

18 ~~135~~. The device according to claim ~~119~~ 1 wherein at least one reflecting mirror is arranged between the pinhole aperture and the detector device.

19 ~~136~~. The device according to claim ~~119~~ 1, further comprising

e) an additional laser beam generation device for generating an additional laser beam with a wavelength different from the first wavelength,

f) an additional focusing device for focusing said additional laser beam onto the measuring volume wherein the additional laser beam is such highly focused within the measuring volume that in essence it exclusively covers the measuring volume,

g) an additional detector device for detecting fluorescence radiation generated due to excitation of one or more molecules, molecular complexes or molecular fragments, and

h) a correlator unit which is connected with the two detector devices.

20 ~~137~~. The device according to claim ~~136~~ 19, further comprising

i) a T-shaped support with a first supporting arm and a second supporting arm connected therewith and running perpendicular to the first supporting arm,

j) two holding devices arranged at the ends at the faces of the second supporting arm for axial guiding and holding of optical elements ~~(lens, filter, mirror, detector)~~ for the



90

two laser beams and the two fluorescence radiations wherein the focused laser beams impinge on a glass slide bearing the measuring volume and being separably arranged between the two ends at the faces of the second supporting arm and held by them, wherein the two holding devices can be moved synchronously relative to their respective ends at the faces of the second supporting arm in a direction of a longitudinal extension thereof, the two holding devices are extended in a direction of extension of the first supporting arm, and the two laser beams can be deflected by deflecting mirrors through optical openings out of an inside of the first supporting arm onto the optical elements for the laser beams held at the holding devices.

138. The device according to claim 136, further comprising

i) a T-shaped support with a first supporting arm and a second supporting arm connected therewith and running perpendicular to the first supporting arm,

ii) two holding devices arranged at the ends at the faces of the second supporting arm for axial guiding and holding of optical elements (lens, filter, mirror, detector) for the two laser beams and the two fluorescence radiations wherein the focused laser beams impinge on a glass slide bearing the measuring volume and being separably arranged between the two ends at the faces of the second supporting arm and held by them,

wherein the two holding devices can be moved synchronously relative to their respective ends at the faces of the second supporting arm in a direction of a longitudinal extension thereof, the two holding devices are extended in a direction of extension of the first supporting arm, and the two laser beams can be deflected by semitransparent mirrors through optical openings out of an inside of the first supporting arm onto the optical elements for the laser beams held at the holding devices.